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Antistreptolysin O and Anti-Deoxyribonuclease B Titers: Normal Values for Children Ages 2 to 12 in the United States

Edward L. Kaplan, MD*; Constance D. Rothermel, PhD‡; and Dwight R. Johnson, BS*

ABSTRACT. *Background.* **Measurement of antibodies to the extracellular antigens produced by group A streptococci, antistreptolysin O (ASO) and anti-deoxyribonuclease B (anti-DNase B), is often necessary to confirm a clinical diagnosis of a previous group A streptococcal infection, especially in patients suspected of having a nonsuppurative sequel to this infection. Age is among several factors that may influence antibody levels in children. Thus, in contrast to adults, what is considered a normal titer for one age group (infants) is not appropriate for another (older children). Age-related "normal" values for ASO and anti-DNase B are provided in the package inserts of commercially available kits; however, there are no recent comprehensive data to validate such values.**

Objective. **Using sera from 1131 children (from 23 states) ages 2 to 12 years, we determined age-specific geometric mean titers (GMT) and upper limits of normal (ULN) of ASO and anti-DNase B.**

Methods. **ASO and anti-DNase B titers were measured by conventional laboratory methods.**

Results. **Children 7 years of age comprised the largest proportion (14%) of the study population. Approximately two-thirds of the sera were collected during winter and early spring months. For both ASO and anti-DNase B, both GMT values and ULN increased with age. The GMTs for ASO and anti-DNase B for the entire group of subjects were 89 and 112, respectively. The ULN for the entire group for ASO and anti-DNase B were 240 and 640, respectively.**

Conclusion. **The age-specific values for GMT and ULN for this group of children from 23 states were slightly higher than previously reported. These values are likely representative of the pediatric population in the United States and should be of clinical value to physicians, epidemiologists, and clinical laboratory personnel.** *Pediatrics* **1998;101:86–88;** ***group A streptococci, antistreptolysin O (ASO), anti-deoxyribonuclease B (anti-DNase B), upper limits of normal, streptococcal antibody.***

ABBREVIATIONS. ASO, antistreptolysin O; anti-DNase B, anti-deoxyribonuclease B; GMT, geometric mean titer; ULN, upper limits of normal.

From the *World Health Organization Collaborating Center for Reference and Research on Streptococci, Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota; and ‡Roche Laboratories, Nutley, New Jersey.

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Address correspondence to: Edward L. Kaplan, MD, Department of Pediatrics, Box 296 Mayo Building, University of Minnesota Medical School, 420 Delaware St SE, Minneapolis, MN 55455.

The diagnosis of group A streptococcal infections is not always possible by clinical history or recovery of the organism. This is especially true when considering the diagnosis of nonsuppurative sequelae of group A streptococcal upper respiratory tract infection, acute rheumatic fever, and poststreptococcal glomerulonephritis. Evidence of a host immune response to group A streptococcal antigens is thus required to confirm the diagnosis. This was initially emphasized in 1965, when the "revised" Jones criteria for the diagnosis of rheumatic fever for the first time introduced the confirmatory role of streptococcal antibody titers in providing evidence of a previous group A streptococcal infection.[1]

The usefulness of streptococcal antibody data may be diminished if physicians or laboratory personnel are unable to knowledgeably interpret the levels of antistreptolysin O (ASO) or anti-deoxyribonuclease B (anti-DNase B). Multiple variables (eg, site of infection,[2] time since the onset of infection,[3] and age[4]) have been shown to influence streptococcal antibody levels. In addition, an incomplete understanding of the kinetics of these immune responses may complicate the interpretation. Clinical microbiology and immunology laboratories often use interpretive criteria suggested by manufacturers of commercial antibody test kits. Because such "normal" levels may only reflect appropriate titers for adults (almost always lower than for children), correct interpretation of titers in children can be problematic.

A review of the literature failed to reveal recent comprehensive studies reporting age-related antibody titers to two group A streptococcal antibodies, ASO and anti-DNase B. Accordingly, we report here age-related mean and upper limits of normal ASO and anti-DNase B titers for a group of more than 1000 children between the ages of 2 and 12 years.

MATERIALS AND METHODS

The sera analyzed here were collected as part of a large clinical trial, the results of which will be reported elsewhere. Sera were obtained between January 1994 and March 1995 from 1131 children (ages 2 to 12 years) residing in 23 states in the United States. These children presented with signs and symptoms of acute-onset pharyngitis (as defined by published guidelines of the Infectious Disease Society of America[5]). All of these 1131 children had positive throat cultures at the initial visit. None of the subjects developed nonsuppurative complications.

Written informed consent for enrollment had been previously obtained. Sera were obtained at the time of the initial acute visit and again 1 month later. The antibody results reported here include only those for the sera obtained at the initial acute visit. The sera were kept frozen at −20°C before testing.

ASO and anti-DNase B titers were determined by techniques

previously described.[6–8] Control sera with established ASO and anti-DNase B titers were included in each test run. For any set of determinations for which a control serum was more than one dilution increment (0.1 log) different from its established value, results from all sera studied for that set were discarded and the determination repeated. Sera were identified only by patient number and the date obtained. Antibody data were recorded in a computerized file for subsequent analysis.

Geometric mean titers (GMT) were calculated and upper limits of normal (ULN) were determined by separating the upper 20% from the lower 80% of the group distribution as previously described.[9]

RESULTS

The age distribution of the 1131 subjects is shown in the tables. The single largest age group was the 7-year-olds (14% of the total).

Because streptococcal antibody titers may vary according to the month when the serum is obtained due to the seasonal epidemiology of group A streptococcal infections,[4] the distribution of patients according to the month the initial serum sample was obtained was examined (data not shown). As would be expected, the largest number of subjects presented during the winter and early spring months. However, we found no significant difference in geometric mean ASO or geometric mean anti-DNase B titers for acute sera for patients enrolled during these months and those enrolled during the remainder of the year (data not shown).

Table 1 shows the GMT (expressed both as logarithms and in Todd units) and the ULN for ASO titers by age. The GMT for each age group increased from 1.7 log units (less than 100 Todd units) for 2- to 4-year-olds to a titer of 2.15 log units (140 Todd units) for 12-year-olds. This age-associated rise in geometric mean titers has been observed in other populations.[4]

The ULN for ASO titer for this pediatric population ranged from a low of 2.08 log units (120 Todd units) for 3- and 4-year-olds to a high of 2.5 log units (320 Todd units) for the 10- to 12-year-olds. The GMT for the entire group of 1131 children was 1.95 log units (89 Todd units); the ULN was 2.38 log units (240 Todd units).

Similarly, GMT and ULN for anti-DNase B were determined (Table 2). The GMT for the entire group of 1131 was 2.05 log units, slightly greater than 100 standard units. The GMT for each age group also increased with age from 1.5 log units (less than 50 standard units) for 3-year-olds to approximately 2.3 log units (220 standard units) for 12-year-olds.

ULN for anti-DNase B ranged from less than 1.8 log units (60 standard units) for the 3-year-olds to approximately 2.7 log units (480 standard units) for 12-year-olds. The ULN for this entire group was 2.81 log units (640 standard units).

TABLE 1. ULN for ASO Titers by Age

Age (y)	N (%)	Geometric Mean Titer ASO		ULN ASO	
		Log	Todd Units	Log	Todd Units
2	27 (2.4)	1.72	52	2.20	160
3	51 (4.5)	1.72	52	2.08	120
4	81 (7.2)	1.72	52	2.08	120
5	122 (10.8)	1.75	56	2.20	160
6	146 (12.9)	1.86	72	2.38	240
7	161 (14.2)	1.94	87	2.38	240
8	131 (11.6)	2.04	110	2.38	240
9	135 (11.9)	2.07	117	2.38	240
10	109 (9.6)	2.10	126	2.51	320
11	87 (7.7)	2.11	129	2.51	320
12	81 (7.2)	2.15	141	2.51	320
Total	1131 (100)	1.95	89	2.38	240

Abbreviations: ULN, upper limits of normal; ASO, antistreptolysin O.

TABLE 2. ULN for Anti-DNase B Titers by Age

Age (y)	N (%)	Geometric Mean Titer Anti-DNase B		ULN Anti-DNase B	
		Log	Units	Log	Units
2	27 (2.4)	1.66	46	2.38	240
3	51 (4.5)	1.47	30	1.78	60
4	81 (7.2)	1.69	49	2.38	240
5	122 (10.8)	1.76	58	2.51	320
6	146 (12.9)	1.88	76	2.68	480
7	161 (14.2)	2.10	126	2.81	640
8	131 (11.6)	2.22	166	2.81	640
9	135 (11.9)	2.27	186	2.81	640
10	109 (9.6)	2.22	166	2.81	640
11	87 (7.7)	2.31	204	2.90	800
12	81 (7.2)	2.34	219	2.68	480
Total	1131 (100)	2.05	112	2.81	640

Abbreviation: ULN, upper limits of normal.

DISCUSSION

The recent apparent resurgence of serious group A streptococcal infections and their sequelae has emphasized the necessity for accurate clinical diagnosis and laboratory confirmation of group A streptococcal infections for both individual patients and for public health analyses. Although there have been recent reports of streptococcal antibody titers in adults and also from other parts of the world,[10,11] we are unaware of recent similar data from children in the United States. The sera collected from these children provided a unique opportunity for determination of ASO and anti-DNase B levels in a large pediatric population with a broad geographic distribution.

It might be argued that because these patients presented with signs and symptoms of acute upper respiratory tract infection and were documented to have group A streptococci in their upper respiratory tracts at the time the initial sera were obtained, their antibody titers could be higher than those of other children. Although this is a remote possibility, we believe this was unlikely. These patients were enrolled in a study because of acute onset of pharyngitis. The mean time between the onset of symptoms and obtaining the initial serum sample was only 1.77 days. Thus, with such a short time interval it is extremely unlikely that these initial antibody titers could have risen significantly. ASO titers reach a maximum at about 3 to 6 weeks after infection, and anti-DNase B titers may not reach maximum titers for 6 to 8 weeks.[3] We believe the individuals included in this report represent a group of otherwise "normal" children who developed pharyngitis, and

the antibody titers obtained at time of onset of symptoms represented immediate preinfection levels.

Whether this group of more than 1000 children was contaminated with streptococcal "carriers" who had harbored the organism for a period of time and then became ill with signs and symptoms of an acute nonstreptococcal upper respiratory tract infection is difficult to determine with certainty. Carriers have been recognized to have relatively high initial titers.[7,12] Whether or not carriers were unintentionally included in this study group would not make a difference in our conclusions, because all normal pediatric populations contain "carriers".[13]

Admittedly, it would have been optimal also to have studied a cross-section of asymptomatic children within this age range and to compare their antibody titers with the values obtained from this present group. However, the difficulties in successfully undertaking and completing such an evaluation are obvious.

Another factor that might conceivably influence streptococcal antibody titers is the effect of antimicrobial treatment. It has been reported that treatment with antimicrobial agents may reduce the magnitude of the antibody response to group A streptococcal extracellular antigens.[14,15] However, these children had not been treated for their illness before the acute sera were obtained and therefore this would not influence the initial titers.

The antibody titers that we reported, especially those for ULN, are somewhat higher than we have reported previously.[4] We do not believe this represents laboratory artifact because the control sera used for each of the two antibody tests are essentially the same used in studies from this laboratory for the past 30 years.

For both the ASO and anti-DNase B titers, both the geometric mean and the ULN for 2-year-olds were equal to or higher than the corresponding levels for 3-year-olds. We believe this to be an artifact based upon the relatively small numbers of 2-year-olds included in the analysis.

In summary, these data provide age-related GMT and ULN for ASO and anti-DNase B titers in a large group of children between the ages of 2 and 12 years. It is not uncommon for both laboratory personnel and physicians to misinterpret streptococcal antibody titers because of a failure to realize that children will, on average, have higher titers than the adult values listed as "normal" in manufacturers' package inserts. Thus, these data can prove helpful for clinicians, laboratory personnel, and epidemiologists in documenting a preceding group A streptococcal infection. It must be recognized, however, that these values are for children in the United States. Because specific ULN and GMT may vary for children living elsewhere, establishment of standard values in those situations will require additional studies.

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